

# Centrosomes and cancer: lessons from a TACC

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The recent discovery that many cancer cells have centrosomal abnormalities suggests a link between centrosomes and cancer. Members of the transforming acidic coiled-coil (TACC) family of proteins have been implicated in cancer and are concentrated at centrosomes, where they regulate microtubule stability. I discuss a model of how the TACC proteins might contribute to cancer. This model predicts that defects in TACC function can make important contributions to the development of cancer but are unlikely to be the primary cause of cancer. The model might also apply to several other centrosomal proteins that have been linked to cancer.

Centrosomes are the main microtubule organizing centres (MTOCs) in animal cells [1,2]. Among other things, they help to organize the poles of the mitotic spindle that is responsible for partitioning the chromosomes equally between the two daughter cells during cell division (Fig. 1a). Although some animal cells can organize their spindles without centrosomes [3], when centrosomes are present, they seem to be dominant [4]. It is important, therefore, that cells regulate centrosome behaviour to prevent chromosomes segregating abnormally during mitosis (Fig. 1b).

## 'Can recent discoveries about the transforming acidic coiled-coil (TACC) family of proteins shed new light on the link between centrosomes and cancer?'

Because of their importance in chromosome segregation, a link between centrosomes and cancer has long been suspected [5]. Many, if not all, cancer cells exhibit genetic instability, where chromosomes or fragments of chromosomes are lost or gained by a cell. This phenomenon seems to be essential for the development of cancer [6], so the recent finding that many cancer cells have centrosomal abnormalities has generated much interest [7–11]. It is unclear, however, whether centrosome defects might be a primary cause of cancer or whether they arise only as a secondary consequence of some other defect. The loss of a checkpoint control that monitors the fidelity of mitosis, for example, could also lead to both genetic instability and centrosomal abnormalities. Thus, the

nature of the link between centrosomes and cancer remains unclear. Here, I discuss how recent experiments are revealing tantalizing clues as to how one family of centrosomal proteins might contribute to the development of cancer.

### The TACC family of proteins

Members of the transforming acidic coiled-coil (TACC) family of proteins have all been implicated in human cancer [12,13]. TACC1 was identified in a search for genes located in 8p11, a chromosomal region that is amplified in 10–15% of human breast cancers [14,15]. The overexpression of TACC1 transforms primary mouse cells in culture, supporting the idea that amplification of TACC1 might contribute to cancer [13]. A database search revealed two other proteins in the human genome (TACC2 and TACC3) that were related to TACC1 in a ~200-amino-acid C-terminal region (the 'TACC domain'), which is predicted to form a coiled coil. Outside of this domain, the TACC proteins share no obvious homology (Fig. 2a), but TACC2 and TACC3 both map to chromosomal regions that are disrupted in some cancers, and TACC3 levels are upregulated in several cancer cell lines [12,13]. Taken together, these findings suggest that the overexpression of the TACC genes might be linked to cancer.

Surprisingly, TACC2 has also recently been identified as a potential tumour suppressor. In a search for genes differentially expressed between malignant mammary epithelial cells and their immediate nonmalignant progenitors, TACC2 (called AZU-1 in this study) was often found to be downregulated as the cells progressed from a benign to a malignant phenotype [16]. Increasing the expression of TACC2/AZU-1 in the malignant cells reverted them to a benign phenotype, both *in vitro* and *in vivo*, providing compelling evidence that TACC2/AZU-1 can function as a tumour suppressor. How can TACC proteins potentially function as both transforming and tumour-suppressing proteins?

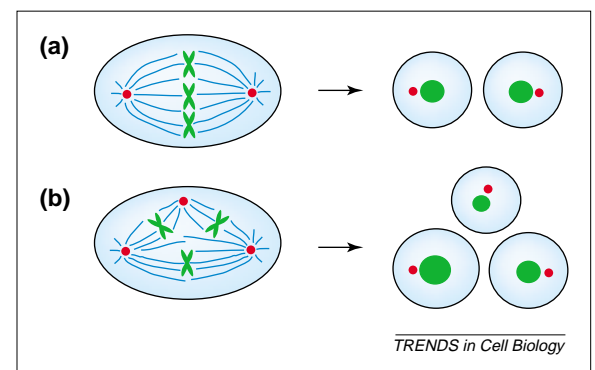


Fig. 1. The importance of centrosomes in animal cell mitosis. (a) A normal mitotic cell with two centrosomes (red) forms a bipolar spindle (blue) and segregates its chromosomes (green) equally to the two daughter cells. (b) In a cell with centrosomal abnormalities (in this case a single extra centrosome), the spindle forms abnormally, leading to the missegregation of chromosomes.

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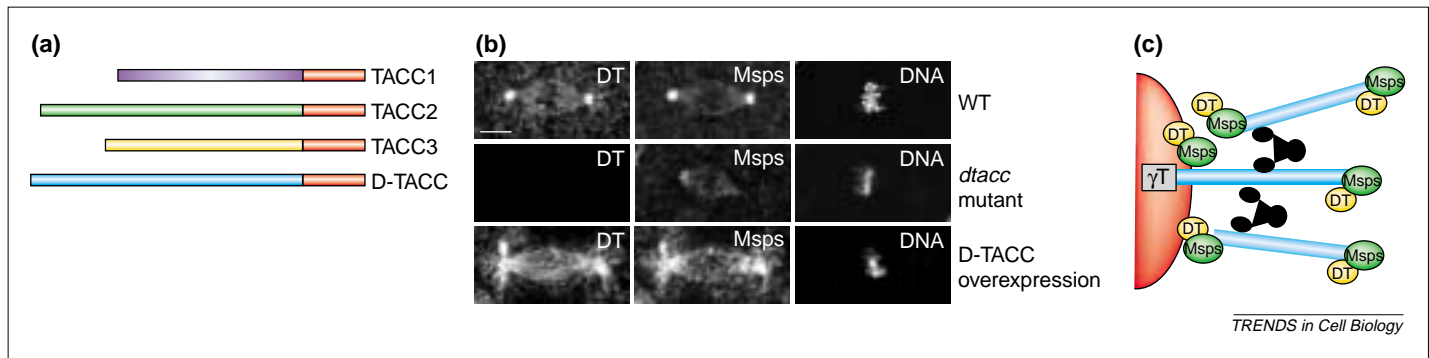


Fig. 2. (a) A schematic representation of the three known human transforming acidic coiled-coil (TACC) proteins and *Drosophila* TACC (D-TACC). The conserved TACC domain is shown in red. (b) The function of D-TACC in *Drosophila* embryos. In a normal (WT) embryo in metaphase (top panels), both D-TACC (DT) and Minispindles (Msp) are strongly concentrated at centrosomes and more weakly concentrated on the mitotic spindle. In a *dtacc* mutant embryo in metaphase (middle panels), D-TACC is not detectable; Msp can still bind to spindle microtubules (which are shorter than normal) but are no longer concentrated at centrosomes efficiently. In an embryo overexpressing D-TACC (bottom panels), extra D-TACC is concentrated around the centrosomes; this recruits extra Msp, and spindle microtubules are stabilized. Bar, 10  $\mu$ m. (c) A model of how D-TACC and Msp might cooperate to stabilize centrosomal microtubules. D-TACC (yellow) recruits Msp (green) to the centrosome (red), where they stabilize centrosomal microtubules by binding to the minus-ends of microtubules that have been released from their nucleation sites (gray), and by loading onto the plus-ends of the microtubules as they grow out from the centrosome. Microtubule minus-end-directed motor complexes such as dynein/dynactin (black) help to focus the released microtubules around the centrosome.

A clue to the normal function of the TACC proteins came with the discovery that *Drosophila* TACC (D-TACC) is a centrosomal protein [17]. If D-TACC function is perturbed in fly embryos, astral and spindle microtubules are shorter than normal, suggesting that D-TACC normally stabilizes centrosomal microtubules. D-TACC is now known to interact with Minispindles (Msp) [18], the *Drosophila* homolog of XMAP215. Members of the XMAP215 family of proteins bind directly to microtubules, stabilizing them by promoting their polymerization [19–24]. Surprisingly, although members of this family of proteins promote mainly plus-end microtubule growth, they are normally concentrated at centrosomes, where the minus ends of the microtubules are clustered. In *dtacc* mutant embryos, however, Msp is no longer efficiently concentrated at centrosomes, and centrosomal microtubules are destabilized. Conversely, if D-TACC is overexpressed in otherwise normal embryos, the extra D-TACC recruits extra Msp to the centrosomes and centrosomal microtubules are stabilized [18] (Fig. 2b). D-TACC is also required to efficiently recruit Msp to the poles of the acentrosomal meiotic spindles in oocytes, suggesting that these proteins can work together to regulate microtubule behaviour even when centrosomes are not present [25].

We have proposed that D-TACC and Msp cooperate to stabilize centrosomal microtubules in *Drosophila* embryos by binding both to the minus-ends of microtubules that have been released from their centrosomal nucleation sites and to the plus ends of the microtubules as they grow out from the centrosome (Fig. 2c) [18]. In support of this model,

both D-TACC and XMAP215 interact preferentially with microtubule ends [17,22], and small dots of Msp–green-fluorescent protein (GFP) and D-TACC–GFP can be seen oscillating to and fro from the centrosome, as though attached to the growing and shrinking plus ends of the centrosomal microtubules [18]. An attractive feature of this model is that centrosomal microtubules are preferentially stabilized in a cell, as only centrosomal microtubules are exposed to a high concentration of the D-TACC–Msp complex.

Do the human TACCs also stabilize centrosomal microtubules, and could this explain the link between the TACC proteins and cancer? All of the human TACCs are now known to interact with both centrosomes and the human homolog of Msp, the colonic–hepatic tumour-overexpressed gene (ch-Tog) [18,26] that, as its name implies, has also been linked to cancer. Surprisingly, however, the human TACC proteins are not all localized in the same way in human cells. In HeLa cells, TACC1 stains centrosomes only during mitosis, TACC2 stains centrosomes throughout the cell cycle, whereas TACC3 stains a much more diffuse area around the centrosome and on the spindle, but again only during mitosis. Thus, the interaction of the TACC domain with centrosomes and microtubules seems to be dependent on its context within the TACC protein and to be regulated during the cell cycle.

The discovery that D-TACC interacts with, and is an *in vitro* substrate of, the Aurora A kinase (AAK) might explain how the TACCs are differentially localized [27]. AAKs are conserved centrosomal proteins that are essential for mitosis and have been implicated in human cancer [28,29]. If AAK function is perturbed in *Drosophila* embryos, centrosomal microtubules are abnormally short, and D-TACC is no longer efficiently concentrated at centrosomes in mitosis. Moreover, AAK also interacts with TACC3 in human cells, suggesting that phosphorylation by AAK might regulate the localization of the TACC proteins in both flies and humans.

Unlike D-TACC in flies, when human TACCs are overexpressed in HeLa cells they form large, highly ordered polymers in the cytoplasm that also contain ch-Tog [18,26]. These polymers also form when any of

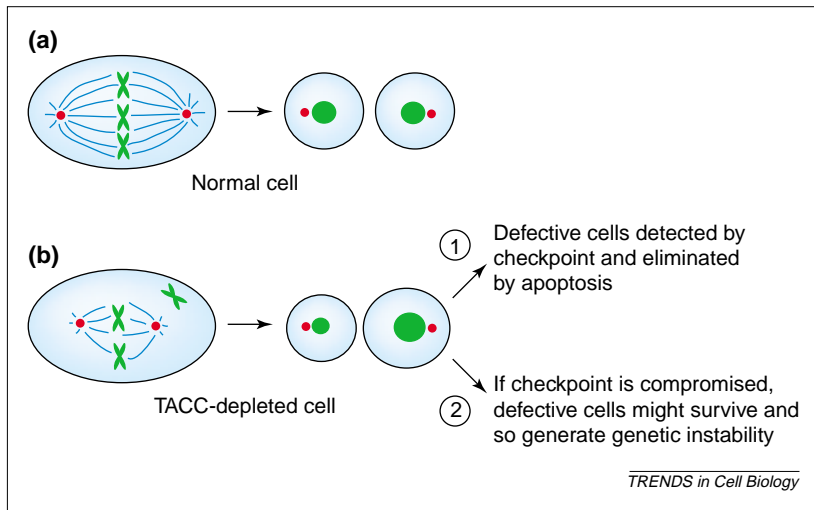


Fig. 3. A model of how the TACCs could contribute to cancer. (a) Cell division in a normal cell. (b) Cell division in a cell where TACC levels are too low. The spindle microtubules are less stable than normal, and they have failed to attach properly to one of the chromosomes, activating the spindle assembly checkpoint. In many cell types, this checkpoint is eventually overridden, and the cell can exit mitosis. In most cells, a p53-dependent checkpoint detects that mitosis has occurred abnormally and triggers apoptosis (pathway 1). If, however, the cell is defective in this checkpoint, the abnormal cell can survive and can go on to generate further genetic instability (pathway 2).

the TACC domains alone are overexpressed in HeLa cells. The ability to form large polymers is unusual, and it raises the intriguing possibility that TACC proteins could contribute to the centrosomal matrix. As these polymers sequester the overexpressed TACC proteins, no extra protein is recruited to centrosomes, so the role of TACCs in stabilizing centrosomal microtubules cannot be addressed. Fortunately, the TACC3 polymers partially disassemble in mitosis and the extra TACC3 then accumulates at centrosomes. As in flies, the extra TACC3 recruits extra ch-Tog to centrosomes, and this stabilizes the centrosomal microtubules [18]. Thus, the TACC and XMAP215/ch-Tog proteins seem to cooperate to stabilize centrosomal microtubules in both flies and humans.

#### The TACC proteins and cancer

The above observations suggest a plausible model for how TACCs might be linked to cancer in humans. In flies, increasing or decreasing D-TACC levels in embryos stabilizes or destabilizes centrosomal microtubules, respectively. In either case, spindle function is impaired and many embryos die early in development as a result of mitotic defects. If human TACCs have a similar function, then alterations in TACC protein levels could result in spindle defects, which could contribute to cancer by increasing genetic instability. This model would explain how TACCs can act as both transforming and tumour-suppressing proteins.

Recently, the gene encoding TACC3 has been knocked out in mice. These knockout mice die late in embryogenesis with greatly reduced cell numbers due to a large increase in apoptosis [30]. The reasons for the increased apoptosis are unclear, but the

lethality of TACC3<sup>-/-</sup> mice can be partially rescued by deleting one or both copies of p53. An obvious possibility is that the TACC3<sup>-/-</sup> embryos have defective mitotic spindles and this eventually triggers apoptosis via the p53 pathway [31]. Surprisingly, however, TACC3<sup>-/-</sup> p53<sup>-/-</sup> haematopoietic cells show only a modest increase in chromosomal abnormalities [30]. The authors suggest, therefore, that the deletion of TACC3 does not trigger apoptosis by causing mitotic defects but rather that TACC3 interacts more directly with p53, perhaps functioning to keep it inactive during mitosis. Indeed, p53 has recently been shown to be concentrated at centrosomes [32–34].

The exact role of p53 during mitosis, however, remains unclear. In many cells in culture, for example, depolymerizing microtubules with drugs activates a spindle-assembly checkpoint that detects the spindle defect and arrests cells in mitosis. This checkpoint is relatively well characterized [35,36], and it does not seem to require p53 [31]. Eventually, however, arrested cells can exit mitosis in a poorly understood process often termed 'mitotic slippage'. In normal mouse embryonic fibroblasts (MEFs), the cells that slip through mitosis then enter the apoptotic pathway, presumably because a second checkpoint detects that there was a problem during the previous mitosis. In MEFs derived from p53<sup>-/-</sup> mice, however, this checkpoint is compromised; arrested cells that exit mitosis are not eliminated and the cells can re-enter the cell cycle [31]. Importantly, this second p53-dependent pathway can eliminate cells that have transiently arrested in mitosis even if they eventually exit mitosis with no detectable chromosomal abnormalities [33]. Thus, perhaps the TACC3<sup>-/-</sup> mice do have subtle spindle defects, but these only delay mitosis, and the cells can usually complete mitosis without any gross chromosomal abnormalities. This delay, however, is enough to trigger the p53 apoptosis pathway in the next cell cycle. In support of this possibility, we have recently found that partially depleting TACC3 in HeLa cells leads to a delay in mitosis; the spindle microtubules seem to be shorter than normal, and the chromosomes take a long time to align at the metaphase plate (F. Gergely and J. Raff, unpublished).

These findings have important implications for how centrosomal proteins, such as the TACCs, might contribute to cancer in humans. If TACC protein levels were altered in a normal cell, spindle function might be impaired, but a p53-dependent checkpoint would detect that mitosis had not occurred normally and trigger the apoptotic pathway in the following cell cycle (Fig. 3b, pathway 1). If TACC levels were altered in p53<sup>-/-</sup> cells, the cells in which mitosis had occurred abnormally would not be eliminated, thus potentially generating the genetic instability that could contribute to the development of cancer (Fig. 3b, pathway 2). In such a model, alterations in TACC

levels would contribute to the development of cancer only in cells where this p53-dependent checkpoint was already defective. Thus, altering the levels of the TACC proteins is perhaps unlikely to be a primary cause of cancer. I would argue that this is likely to be the case for many other centrosomal/spindle proteins that have been shown to be aberrantly expressed in tumour cells.

Importantly, when considering how the TACCs, or any other centrosomal proteins, might contribute to cancer, it is worth remembering that they might promote cancer in ways that are independent of their function at centrosomes. Maskin, for example, which is the only known TACC protein in frogs, regulates the translation of maternally stored mRNAs in the

frog oocyte [37]. Although maskin is a centrosomal protein, it is not yet clear whether it also has a role in spindle function in frogs.

#### Concluding remarks

In summary, any abnormality in our cells that contributes to genetic instability is likely to promote the progression of cancer once it develops. Alterations in TACC protein levels might promote cancer in this way, and this might be true of any alteration that leads to centrosome/spindle defects. An increase in genetic instability alone, however, might be insufficient to trigger the development of cancer, as many cells normally seem to have a checkpoint that eliminates cells that pass through mitosis incorrectly.

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